Ribi, Edgar 1985

Dr. Edgar Ribi Oral History 1985

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Edgar Ribi

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Oral History Interview

This is an interview with Dr. Edgar Ribi at his office in Hamilton, Montana, August 1, 1985. Electron microscopes, Ph.D. dissertation and postgraduate work are the topics of the interview.

ER: I studied physical chemistry in Bern in Switzerland and when I finished my thesis, I was one of the lucky chosen to go to Sweden for a year to work in Dr. Svedberg, Professor Svedberg's laboratory, the man who developed the ultracentrifuge and very prominent in the field of macro-molecular chemistry. When I came to Uppsala, my project was to study the structure of synthetic and natural fibers and especially the cellulose fiber, because the wood industry was very important in Sweden. I found in the basement of the Svedberg Institute one of the earliest Siemens electron microscopes and that Siemens microscope was given to Svedberg by Mussolini who had gotten it from Hitler as a present and [when the axis broke] Mussolini gave this microscope to the Swedes to Svedberg. I worked with this microscope the best I could. I studied the fine structure of the natural fibers and found I had to disperse it by ultra-sonication in order to prepare a cotton, a thick piece of wood or even a cotton fiber under the microscope unless you disperse it so the electron microscope comes into fine structure. So it disperses this whole ultra-sonication and found microfibers, natural dimers in 100-150 Angstroms about 100 cellulose molecules joined and formed fiber. The same principle found with nylon and this is also synthetic fibers a natural colloidal phenomenon. But Svedberg thought it might be an artifact because I had been using the ultra-sonication and he told me I was to have a thin section of the wood where I can see unaltered fine structure. At the time the best electron-microscopist who knew how to make sections was Dr. Wycoff of the NIH, so Svedberg gave me a fellowship to go to Wycoff and make sections of the wood. Wyckoff said that will not be possible. We have no microtomes to make such thin sections out of wood. The possibilities that you can cut these sections if you use glass knives. So I found a way and made glass knives from broken glasses from the windows of street cars and in order to make thin sections, I was using an old Spencer microtome which made it so they would not move a micron, they do not move at all. I would embed the pieces of wood in some plastic and then, puff from my pipe, one puff and then one turn and the expansion from this little temperature increase would be sufficient to make me the section of wood. It has not been repeated until this time, this wood single section. Wyckoff was very happy about this, he wanted to keep me at the lab in Bethesda. But while I was studying at Bethesda, I used to make use of all the different laboratories. This was unusual. You'd be in one building and not in the other building but for me, I looked for the tools and techniques. I did electron microscopy in Building 4 with Wyckoff, the chemistry I did over in Building 2 and the ultracentrifugation in the old Cancer Institute, the ultra-sonication in the attic of Building 7 and certain procedures in the old Building 5. So I knew pretty soon everybody there. It was very nice time. When I had those sections, I finished the work. I went back to Sweden and after a short stay there, I got back to work with Tiselius on electrophoresis to work this technique. Meanwhile at the Rocky Mountain Laboratories, they had a re-organization and Dr. Larson was to become director and introduce some other disciplines and they wanted to introduce an electronmicroscope to study his so-called soluble antigens. When I came here to Hamilton, I had never had too much contact with immunologists, bacteriologists, microbiologists. After two months, the microscope was working and I wanted to have something to do. I noticed the interaction of antigen-antibody. When you make a vaccine, they would pour some phenol or formalin or ether over the bacteria and inject the killed organisms. Yet under the electronmicroscope I see this nice cell walls encapsulating protoplasm and figured, yeah, maybe the immunologic goodies which produce these immune responses are probably on the surface rather than within. I suggested to Larson, why don't we make some cell walls by shaking the tularemia bacteria, break the cells up, let the cell protoplasm extrude and use the cell wall for vaccine. Larson told me “I know you are a nice guy and I am going to waste some money and mice on you”. So I made cell walls and he made a nice quantitative dose-response effect from those things. There were no toxicity observed and the animals were protected against challenge with the virulent tularemia. That started the role to dissect the microorganism to study on molecular levels what are, how the bug is producing the disease phenomenon. Only then would you understand what are the immune phenomena and then would you know when the infectious disease process on a molecular level can you intelligently also produce a vaccine. That you would separate the toxins from the protective antigens and allergens and so on, and this work became my project with wholehearted support from Larson to get a group together that could work interdisciplinarly, namely the chemist, the immunologist, bacteriologist as well as the veterinarian. After a couple of years when we had the tularemia bacteria, then we stocked the Munoz (*Haemoph ilus*) *pertussis bacteria*. We made cell walls. This is a very toxic vaccine.

Interviewer: Is this pertussis?

ER: On the cell was they were protective and the toxin will not be as toxic. We went and wanted to have prepared the vaccine and non-living vaccine against tuberculosis.

Interviewer: Right

ER: We came to the *tubercle bacillus* this is a (bacciphagi?, BCG) organism. That was at the time when Dr. Smadel came to the lab for a review. He was very excited about what we had been doing so far, but he said, and I told him when I shake this culture, this *tubercule bacillus*, it breaks up but it comes to a bubble like consistency. You cannot make nice cell walls when you shake it; you just make bubbles. I said to Smadel that I thought there is a possibility instead of shaking I would like to use the principle of rapid pressure and reversal, namely the pressure cell to put the bugs under high pressure and release of the orifice of the needle valve so that the bug comes out, and collapses. It is extruded that I wouldn't have to shake it and I would like to buy a press cylinder. Smadel said, look, that won't work, you not going to do that. I said, look, I am not married to you, I can always go back and make this in Switzerland but I want to try that. So, that was when the Montana Tuberculosis Association met. The meeting was in Missoula and I went there and told them, would you mind giving me $550 to buy a press cylinder and I'm going to try that. And they did. So I used the press, and I used high pressure. When I looked under the microscope I saw some cell walls, but the rest didn't look good. The protoplasm was all coagulated because of the heat effect. So I went back to an old principle used in electronmicroscopy, the carbon dioxide chamber and I cooled the orifice of the needle valve down to -50C and applied the pressure and out came this beautiful phenomenon and here are these cell walls. Just a ghost left. Okay? That's what I ended up. They gave me a medal for it.

Interviewer: For the refrigerated cell fractionator?

ER: Yes for the refrigerated cell fractionator. So we made tubercle cell wall. We found that when you cover teensy little oil droplets with these cell walls, that was much more effective than was the Freund type adjuvant with 50% oil. The reason for the oil in water instead of the water in oil emulsion was Larson was telling me, you know, with oil, something is extracted. I said, no, nothing is extracted with oil. I can prove that to you. I can take some cell walls and just enough oil to make a paste and then I grind them and spin them out. Then I would have no extraction effect and that turned out to be the most important point, namely the oil in water emulsion. We had only 1% oil. Droplets in the microsome has been covered with the cell walls, injected into mice and to monkeys, and then challenged with T.B., it was beautiful protection. These are the lungs of monkeys which had been challenged without being vaccinated. The lungs of monkeys which had been treated intravenously with the cell wall emulsions and were clean as a whistle. The only trouble is it produced a granulomatous response that was in the lung lining. An at this time these men found, Rapp and Zbar's group of the (National) Cancer Institute, they discovered that when you inject guinea pig line-10 tumors with viable BCG you would have tumor regression, not only tumor regression but also immunity against that tumor, the syngeneic tumor. We figured that maybe our cell walls, if injected in a tumor produces granuloma, it would get rid of the tumor and you would not have all this detrimental effect the viable BCG would have. With a tumor trial with BCG you get suppressor cell activity, you get harmful reactions and you cannot control it. So Dr. Rauscher came our here, the director from the National Cancer Institute, he said okay, I think that is fine. The team Rapp and Zbar should come out here and introduce the line-10 guinea pig tumor. So we injected guinea pigs and the tumor regressed and really true immunity was produced because once the tumor of the guinea pig had regressed you could afterwards challenge it with a little dose of tumor cells, you would have a delayed type hypersensitivity reaction against the tumor and by doing this immunity was produced. This was more effective than the viable BCG. Following the work we took the tubercular protein out, we took the important lipids out. We had the cell wall skeleton, we found out what are the minimum structural units in order to produce regression of the tumor. So we had these so-called cell wall skeleton trehalose di-mycolate that I called P3 and in order to purify trehalose di-mycolate that was sterile, huge macromolecules, 90 carbon, and the method did not exist. So that is when we invented microparticulate cell chromatography. The reason was to purify this trehalose di-mycolate which was a component of the tubercle bacillus cell wall which in combination with the cell wall skeleton would have all the activity of the entire cell wall but it would not have the tubercle protein which could cause an allergic manifestation. So we proved that you don't need tuberculin reactivity in order to get the action of the Freund's type adjuvant. You needed only two components: you needed trehalose di-mycolate and needed the cell wall skeleton. And with these two components, you could stimulate immune responses like the old Freund's type adjuvant. Also you needed only 1% oil in droplets which you could give intravenously. So that is the principle of the mycobacterial adjuvant consists of two components. We could take these two components and combine with any old protein, then you would stimulate immunity against that very protein in adjuvant type specificity. So this then also worked nicely in animals, horses, in cow's eye and horse sarcoid. And that was the first clinical trial done on malignant melanoma; that looked also good. But there was another program that used mycobacteria as adjuvants which were important and other the most powerful adjuvant was the bacterial endotoxin, the endotoxin from the Gram negative bacteria. But that was very adjuvantistic (?) with the mycobacterial adjuvant. The endotoxins were simply too toxic to be used in large animals and in humans, little mice and guinea pigs could tolerate it, but not rabbits and larger animals. So we became very much interested in the endotoxin. We worked on endotoxin and we knew that toxicity and adjuvanticity of the endotoxin was not correlated with toxicity because sometimes the material was not very toxic when some acid treatment was involved and yet we still had the same adjuvant properties. There was still residual toxicity which we couldn't get. We know that endotoxin must have an outside label group. Wenner and I predicted, I think it was back in 1960, and we didn't know what is the score and I was setting out to get the endotoxin taken apart and find out the structure-function relationship. That is what I very badly wanted, because I know once we have the toxicity out from the endotoxin we combine it with this [unintelligible] we have a very good natural adjuvant. One of the best biologic response modifying agent to which the host is primed and so, just a year ago, when I still was at the Rocky Mountain lab, we used the tumor model and we learned more about endotoxin produced using tumor as the model than we used in infectious diseases. That was not well appreciated. They said, you know if you work on tumors, you actually should go to the Cancer Institute, and I said you know the same immune system that is responsible for infectious diseases also is responsible for this including tumors. But I would like to solve the puzzle of the endotoxin and so, as you know, we succeeded to get endotoxin fractionated. We found out all it needed is to get a certain phosphate group, the labile phosphate group get off which then lets the whole molecule still be active but far less toxic, a thousand times less toxic and the explanation may be the same of the ones for organophosphate pesticide. They do have a labile phosphate group and phosphoester cholinesterase brings about the phenomenon. Anyhow, the nice thing is when we had this detoxified endotoxin and we combine it with this material we have extreme fine potentiation of the formation of antibody. We have the enhancement of stimulation of colony stimulating factors; Interleukin 1 and we have tumor necrosis. We have observed we have interferon production, the whole array of the immunostimulants and so, when you inject this combination you get on a natural way all the ingredients that help to stimulate the immune responses be it humoral of be it cell mediated immune responses. The tumors regressed even far better than they regressed with the cell wall skeleton and trehalose di-mycolate. Right now we have the synthetic peptides we have that carry the optimal determinants. There are several synthetic peptides and without the adjuvant, there you go without a titer. If you take the antigen in alum you get 400; if you take the Freund type adjuvant you get 12-fold, but if you use the detoxified endotoxin together with the trehalose di-mycolate we get a titer in thousands. This is now what they have. They can't use the Freund type adjuvant because you can't inject it. You can use alum, that is 6000 for to half-a-million titer. Here is ovalbumin, a generally weak antigen in Freund type complete adjuvant, 50% emulsion oil, titer 1600. You use that detoxified endotoxin, with the trehalose di-mycolate get a titer 100,000 here and 200,000 there. This goes to show you that we anticipate that the modern type vaccines now with genetically engineered proteins or synthetic peptides which they make we can have adjuvant for it. So far, the results have been very very exciting but what we do now is our problem here with to upscale that we can make these larger quantities. This is a real challenge.I always wanted to stop the work the moment we have the endotoxin problem solved so that's what I really wanted. But now we got involved into practical application and now we can to help to produce tumor immunity, this is one thing but the application of a vaccine and other immune stimulant on the whole is even without the antigen is now is very very interesting and puts us into very exciting new area where we can do good.

Interviewer: I think it is very interesting that what would be considered basic research you are now able to do commercially. Do you think this is a development in the last fifteen years or so?

ER: Yes, we always wanted to do basic research but when you see that there is an application, do it. So we went on our own and I was mobilizing all the wealthy names around the country and Montana for field trials and doctors' help. That is important. As you may know, some of my colleagues, they came with me here and we still have nice collaboration with the Japanese, with the people over in Europe and with the industry and so we are here in the mountains. Scientists, but we collaborate with more than thirty right now. It is a lot of fun.

Interviewer: I am sure, and the mountains and scenery adds to it.

ER: This is the life. From the laboratory you can always watch the mountains.

Interviewers: It is beautiful, well, thank you.

End of Interview